



## Development and Validation of a LC-MS/MS Method for the Determination of Rilpivirine in Sprague Dawley Rat Serum and Its Application to Pharmacokinetic Study

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### ABSTRACT

A novel bio-analytical method was developed and validated for the quantitative determination of rilpivirine in rat serum by using the liquid-liquid extraction chromatography and tandem mass spectrometric detection (HPLC-MS/MS). Separation of rilpivirine from the endogenous substances is achieved after liquid-liquid extraction by using HPLC-MS/MS system. Rilpivirine was eluted in isocratic mode with acetonitrile, methanol and 0.1% acetic acid in 5 mM ammonium acetate (20:25:55) at a flow-rate of 0.6 mL/min on Waters, Discovery C18, 50\*4.6 mm, 5µm particle size column. Didanosine was used as the internal standard. The liquid-liquid extraction recovery was found 74% indicates good recovery. The validation results demonstrated that the present method was found to be precise and accurate. The stability tests indicated that the rilpivirine in rat serum is stable for three freeze-thaw cycles at both -20 °C and -70 °C, 18-h ambient storage, 15-day frozen storage at both -20 °C and -70 °C. The results also showed no significant matrix effect (<5.2%). The present method was found to be sensitive and selective at very low levels of linearity range 2-1000 ng/mL, based on a sample volume of 50 µL, with a linear correlation coefficient of ≥ 0.99. The validated method has been successfully applied to support a preclinical pharmacokinetic study.

**Keywords:** Rilpivirine; Bioanalytical; HPLC-MS/MS; Liquid liquid extraction (LLE); Quantification; rat serum.

### 1. INTRODUCTION:

Rilpivirine is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) that is high potent against both wild-type and drug-resistant HIV-1 strains [1]. Consequently, rilpivirine is expected to treat therapy-experienced patients who failed to use current drugs due to the emergence of drug-resistant HIV mutants. In addition, rilpivirine shows a favorable safety profile [2-4]. The recommended dose of rilpivirine is 25 mg (one tablet) once daily in combination with other antiretroviral agents. Rilpivirine is a substrate of CYP3A4 and its pharmacokinetics is likely to be modulated by inhibitors and inducers of these enzymes. To manage these drug interactions and ensure optimal drug efficacy, monitoring plasma rilpivirine concentrations is essential. For this purpose, we developed a method for determining plasma rilpivirine concentrations using LC-MS/MS.

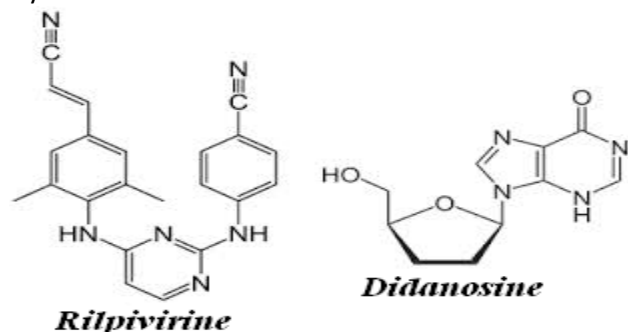
Else *et al* recently determined the simultaneous estimation of plasma rilpivirine concentrations using liquid chromatography tandem mass spectrometry along with other retroviral drugs [5]. In this study, we intended to develop a conventional method for determining plasma rilpivirine concentrations by LC-MS in biological matrices to support the preclinical and clinical studies [6]. Bioequivalence of the Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate Single Tablet Regimen determined in human plasma [7]. The present method has been successfully used to study the pharmacokinetic studies in healthy rats of rilpivirine suspension formulation (5 mg/kg) after oral administration. The main objective of this study is to develop a fine tuning and validated method of a quick, precise and simple analytical method for determining rilpivirine levels in plasma using high-

performance liquid chromatography with MS/MS detection in Sprague dawley rat serum. This method has been developed exclusively to study the pharmacokinetic parameters by non-compartmental design in Sprague dawley rats, which offers the good selectivity and sensitivity than the methods reported earlier.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Acetonitrile (ACN), Methanol, Ethyl acetate, n-hexane and acetic acid of HPLC-grade were purchased from Sigma-Aldrich. Ammonium acetate of SQ grade was purchased from Qualigens. The reference standard rilpivirine was obtained as kind gift sample from Hetero Drugs. (Hyderabad, India). The internal standard (IS) didanosine was purchased from Sigma-Aldrich. The structural formulae of rilpivirine and didanosine are shown in Figure 1. Normal rat serum was collected from Department of pharmacology, Sultan-UI-Uloom College of Pharmacy, Hyderabad.



**Figure-1: Chemical Structures of Rilpivirine and Didanosine (IS)**

### 2.2. Preparation of standard solutions, calibration standards, and quality control (QC) samples

Standards and QC samples were prepared from two separate stock solutions. 5.00 mg of rilpivirine standard was weighed accurately and transferred into a 5 mL of volumetric flask containing 2 mL of methanol and the volume was made up with the same to obtain a concentration of 1.00 mg/mL. This stock solution was used for preparation of calibration standard spiking working samples

The IS working solution (1.00 µg/mL) was prepared in 50 % methanol from IS stock solution. Analyte stock solutions, IS stock solution, and working solutions were stored in polypropylene vials at 2-8 °C in refrigerator. The calibration standards were at concentrations of 2, 4, 15, 50, 300, 600, 800 and 1000 ng/mL and quality control sample i.e., 2.00 (LLOQ), 6.00 (low), 500.00 (mid), 800.00 (high), and 8000 (10 fold dilution) ng/mL were prepared on each day of analysis by spiking in rat serum using calibration standard and quality control spiking\ working samples. The lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) of this method were 2

ng/mL and 1,000 ng/mL, respectively. For evaluation of stability of rilpivirine in rat serum, the QC serum samples at low and high-QC level concentrations were prepared in pre-labeled polypropylene vials (~0.4 mL per vial) and then stored in a freezer maintained at approximately -70 °C and -20 °C for testing sample storage stability under this temperature conditions.

### 2.3. Sample extraction

2 mL of ethyl acetate/n-hexane (50:50, v/v) solvent was added to a vial containing the IS (1µg/ml), 200 µl of 5 mM ammonium acetate and 50 µl serum sample. The mixture was vortexed for 5 min and then centrifuged at 2,500 rpm for 5 min. The upper layer was separated and evaporated dry. The dried material was then reconstituted with 100 µL of mobile phase and 10 µL was injected into the API 4000 LC-MS/MS.

### 2.4. LC-MS/MS conditions

MS/MS detection was performed using a Sciex API 4000 triple quadrupole mass spectrometer with a Turbo Ionspray® ionization source operated in the positive ion mode. The mass spectrometry parameters such as analyte fragmentation pattern and collision energy, etc. were optimized by infusing the analyte and the IS solutions. The LC-MS interface conditions such as gas flows, source temperature, etc. were optimized via tee-mixing the analyte standard solution with the mobile phase at a flow-rate of 0.600 mL/min. All optimized parameters for this method are presented in Table 1.

Source temperature (°C)	550
Dwell time per transition (ms)	200
Nebulizer gas (psi)	40
Heater gas (psi)	45
Curtain gas setting	10
Collision gas setting	15
IonSpray voltage (V)	5500
Declustering potential (V)	95
Collision energy (eV)	28
Collision Cell Exit Potential (V)	12
Resolution for Q1 and Q3 Unit	Unit
Mode of analysis Positive	Positive
Ion transition for rilpivirine,	m/z 367/ 224
Ion transition for IS, Didanosine	m/z 237/136.2

**Table 1. Tandem mass spectrometric parameters for the LC-MS/MS assay.**

### 2.5. Quantification and assay validation

The peak areas of the analyte and the IS were integrated by using *Analyst*® software version 1.4.1. For each analytical run, a calibration curve was derived from analyte/IS peak area ratio against the analyte concentration using a weighed (1/x<sup>2</sup>) linear least-squares regression. The regression equation was then used to calculate the concentration of rat serum samples. During

the method validation, intra-day and inter-day precision (CV %) and accuracy (RE% or percent Bias of theoretical value) were calculated. The presented method was validated following the FDA guidance for bio-analytical method validation [8] for intra-day and inter-day precision and accuracy, linearity, selectivity, sensitivity, dilution integrity, and short-term and long-term sample stability.

**3. RESULTS AND DISCUSSION**

**3.1. Chromatography**

Separation of the analyte from potential interferences in the matrix was achieved on a Waters, Discovery C18, 50x4.6 mm, 5 μm column at 40 °C column oven temperature. An isocratic mobile phase mixture of a) acetonitrile b) methanol c) 0.1% acetic acid in 5 mM ammonium acetate (20:25:55) was used at a flow-rate of 0.600 mL/min. A 1:1 methanol-water solution was used as the injector washing solvent for the prevention of potential carryover from the needle. No carryover from the injection needle was observed during the method validation. In addition, to minimize the evaporation of the reconstituted sample in the auto-sampler vial, the auto sampler cooling system was set at 4 °C. Retention times for both rilpivirine and its IS were approximately 2.5 min and 2.7 min, respectively. The capacity factor *k'* for the analyte peak was about 2.73 (the column dead volume is about 0.93 mL and  $k' = (2.5-0.93)/0.93 = 1.69$ ), indicating that the analyte has a sufficient on-column retention for analysis. The representative chromatograms of ULOQ, matrix blank, and LLOQ samples are shown in Figure 2. The clean chromatogram of the matrix blank obtained from the injection of a matrix blank extract immediately after an ULOQ sample demonstrated that this method had neither injector carryover nor analytical column carryover.

**3.2. Linear curve range and assay sensitivity (LLOQ)**

The linearity of the calibration curve was evaluated from three individual batches on three different days. The linear dynamic range for rilpivirine was from 2.00 to 1,000 ng/mL based on a 50 μL serum. The coefficient of determination (*r*<sup>2</sup>) of the calibration curve was between 0.9978 and 0.9992 and the % bias of mean back-calculated concentrations of standards ranged from -4.50 to 2.50 of the theoretical values (Table 2). Eighteen replicates (six replicates in each of three batches) of the lower limit of quantification (LLOQ) samples were used to evaluate the inter-day precision and accuracy at the low end of the assay range in three separate runs. The inter-day CV% was 7.23% (n = 18) and the accuracy, expressed as percent bias, was 2.06 (Table 3). The representative LC-MS/MS chromatograms of an ULOQ, LLOQ and blank serum sample is shown in Figure 2 (a, b, and c).

**3.3. Precision, accuracy dilution integrity and recovery**

Six replicates of QC samples for each of three consecutive runs were used to evaluate the intra-day and inter-day

precision and accuracy at low-, mid-, and high-QC concentration levels. As shown in Table 3, the intra-day CV (n = 6) was ≤6.71% and the inter-day CV (n = 18) was ≤7.23%. The % bias from the nominal of mean values of intra-day and inter-day assays was between -1.81 to 1.92 and -2.87 to 2.06, respectively. To validate partial-volume assay, a dilution factor of 10 with blank serum was processed with the dilution QC sample (8,000 ng/mL). A 5 μL of the dilution QC was mixed with 45 μL of blank matrix in in pre-labelled polypropylene tubes. Results for the dilution QC (DiQC) samples showed a % bias from nominal of mean value -1.81 and the corresponding CV was 3.48% (n = 6). These results indicate that the present method has satisfactory precision, accuracy and dilution integrity.

Nominal Concentrations (ng/mL)	Back calculated mean concentraion (ng/mL)	Standard deviation	Precision (CV%, N = 3)	% Bias from nominal
2	2.05	0.02	1.13	2.50
4	4.04	0.19	4.72	0.88
15	15.31	0.33	2.18	2.07
50	50.01	2.10	4.20	0.02
300	305.50	5.14	1.68	1.83
600	600.50	15.18	2.53	0.08
800	793.00	9.45	1.19	-0.88
1000	955.00	7.64	0.80	-4.50

Table 2. Back-calculated rilpivirine calibration standards in rat serum

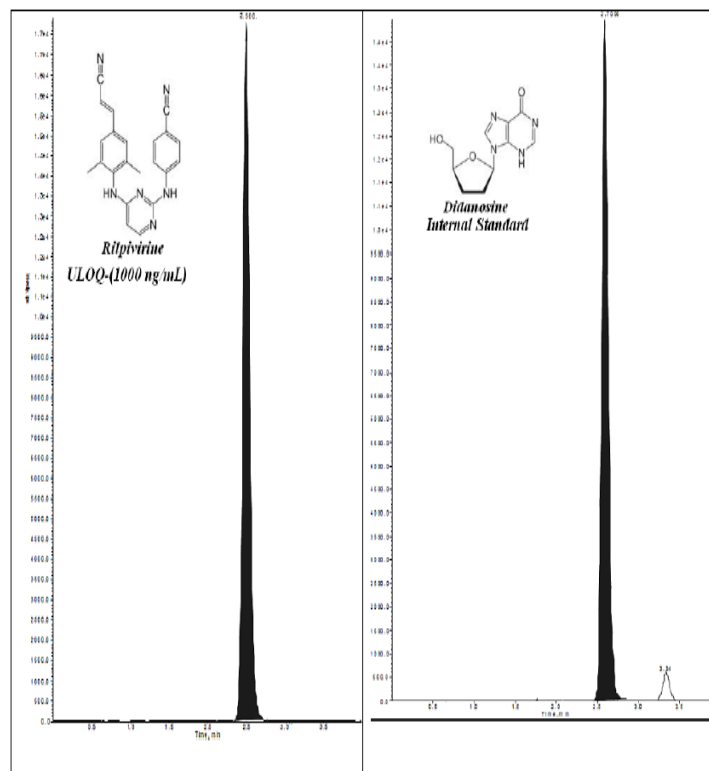


Fig 2 (A) Typical chromatogram of Rilpivirine at ULOQ level contains (1000 ng/mL)

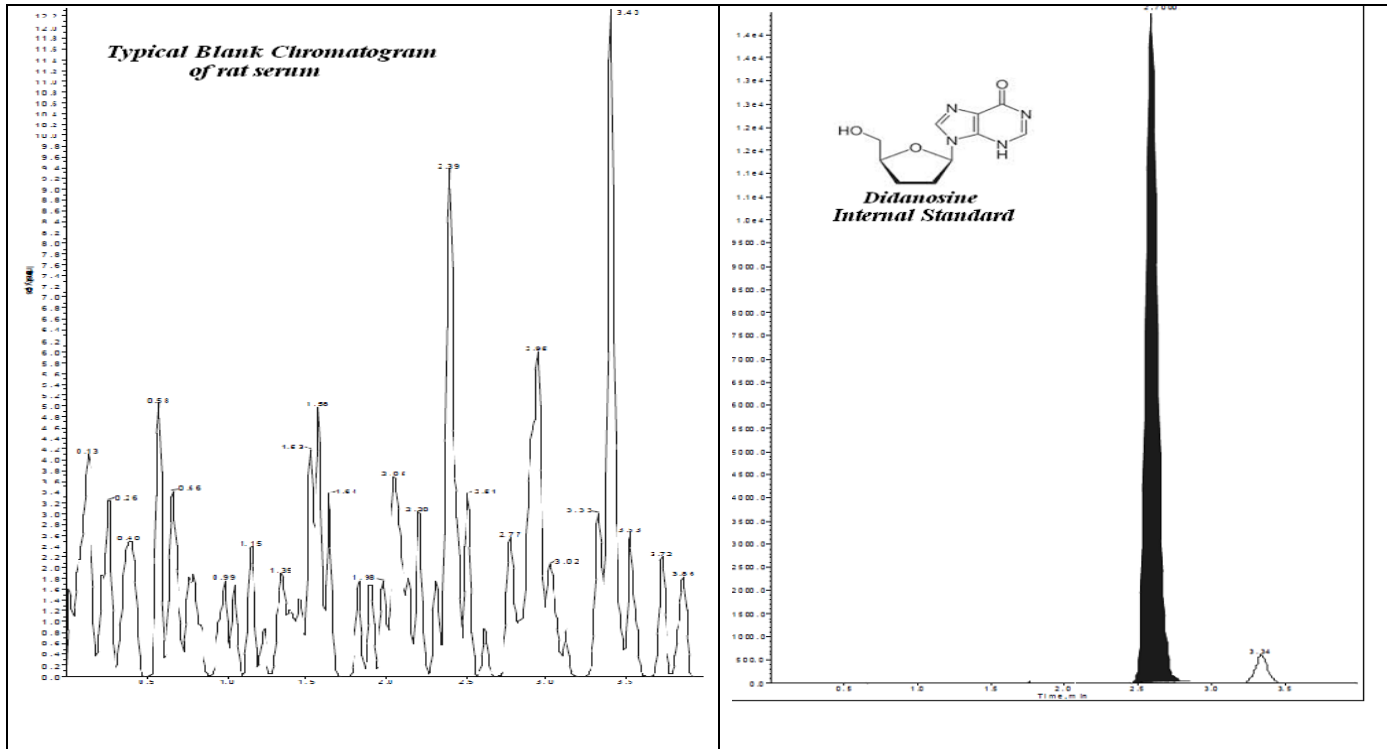


Fig 2(B): Typical blank chromatogram of rat serum sample

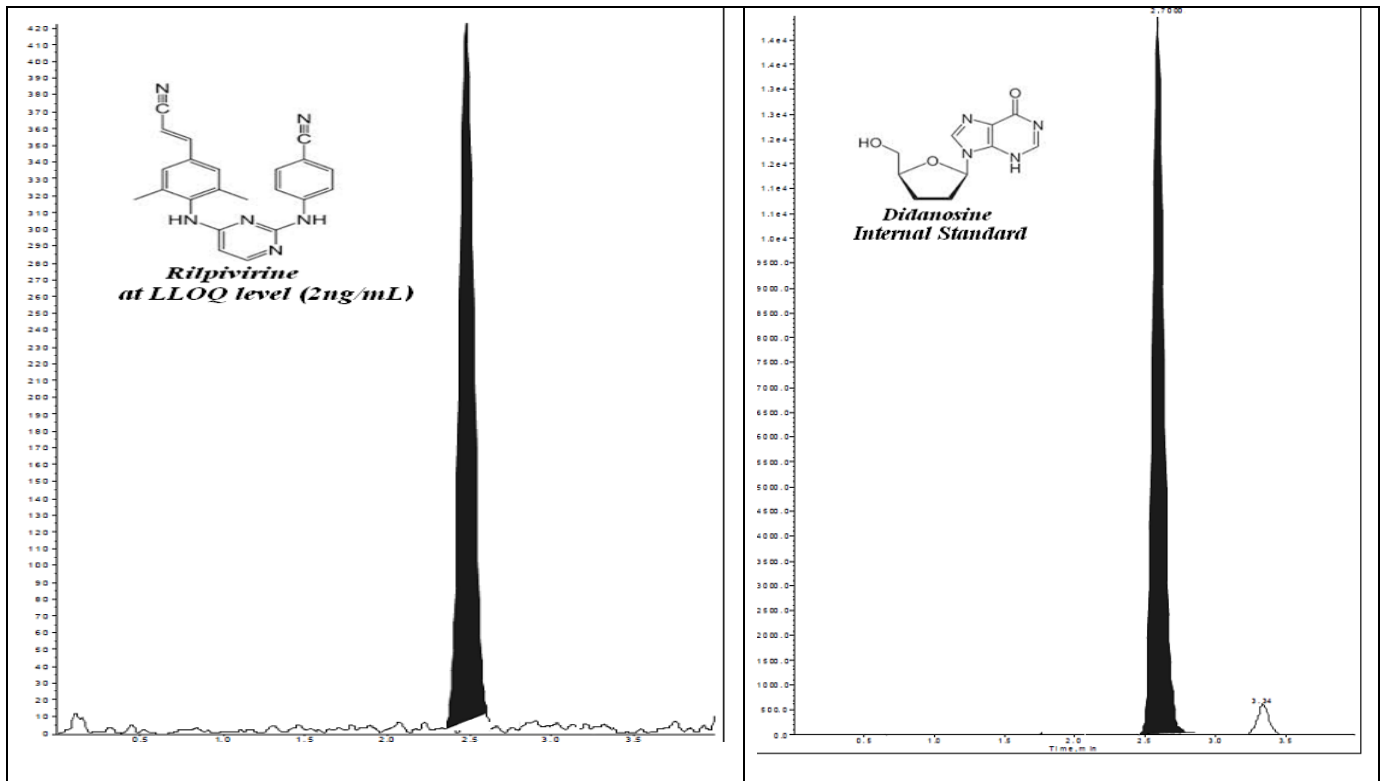


Fig 2(C) Typical chromatogram of Rilpivirine at LLOQ level contains (2 ng/mL)

Figure 2. Typical chromatograms obtained from LLE extracted samples: (A) ULOQ sample (1,000 ng/mL), (B) blank serum sample, and (C) LLOQ sample (2 ng/mL). (The matrix blank sample was injected immediately after an ULOQ, therefore also an indicative of no carryover. Left panel – Rilpivirine; Right panel – Internal standard, IS).

QC sample nominal concentration (ng/mL)	Intra - day					Inter-day			
	LL OQ	LQ C	MQ C	HQ C	DIQC	LL OQ	LQ C	MQ C	HQ C
	2.00	6.00	500	800	8000	2.00	6.00	500.00	800
N	6	6	6	6	6	18	18	18	18
Mean	2.04	6.07	494.31	799.59	7855.02	2.02	5.89	485.65	816.49
%CV	6.71	2.92	1.92	2.87	3.48	6.68	7.23	3.80	2.59
%Bias	1.92	1.22	-1.14	-0.05	-1.81	1.22	1.86	-2.87	2.06

\* 10-fold dilution with the control matrix applied to the DiQC (8,000 ng/mL).

**Table 3. Intra-day and inter-day precision and accuracy studies of quality control samples including LLOQ and the diluted QC\* samples.** Recovery of rilpivirine was evaluated by comparison of mean analyte response in processed LQC, MQC and HQC samples in Sprague Dawley rat serum with mean analyte response from neat solutions at respective levels. Mean recovery values for rilpivirine were 73.57, 76.88 and 79.46 % at low, medium and high quality control levels, respectively. The results are presented in Table 4 (recovery of IS not shown).

QC sample	Precision (CV%, N = 6)	% Recovery
LQC: 3.00ng/mL	8.26	73.57
MQC: 500.06 ng/mL	3.49	76.88
HQC: 791.02 ng/mL	2.53	79.46

**Table 4. Recovery studies of rilpivirine in Sprague Dawley rat serum**  
**3.4. Selectivity and matrix effect**

Selectivity was evaluated by extracting blank matrix from six different lots and comparing the MS/MS response at the retention times of the analyte to the responses of the LLOQ. No significant peak was observed in any of these 6 lots of blank serum samples for analyte and IS. The matrix effect on the responses for both rilpivirine and its IS were investigated by preparing a LQC and HQC level with six different individual lots of serum. The matrix effects were evaluated by comparing the peak area obtained from the post-extraction spiked sample to a pure solution at the same nominal concentration. Matrix effect was calculated as ME (%) = [1 – peak area of post-extraction spiked sample/peak area of pure solution] × 100. With this LLE procedure and chromatographic conditions, the matrix effects for rilpivirine and didanosine were 5.2% and 3.5%, respectively.

**3.5. Sample stability**

Stability tests were conducted to evaluate the analyte stability in stock solutions and in serum samples under different conditions. The ambient storage and freeze-thaw, long-term frozen storage stability at two different temperatures were tested and the results are presented in

Table 5. As shown in Table 5, mean % change from nominal values for ambient storage at LQC and HQC level was -2.28 and 3.77, respectively. Mean % change from nominal values for freeze-thaw stability (3cycles) at -70°C for LQC and HQC level was -0.97 and 2.47, respectively and at -20°C for LQC and HQC level was -1.22 and 0.09, respectively. Mean % change from nominal values for Long-term stability in serum for 15 days at -70°C for LQC and HQC level was -2.22 and 0.02, respectively and at -20°C for LQC and HQC level was -1.06 and 0.34, respectively.

Re-injection of the accepted precision and accuracy batch stored in auto sampler at 4°C was performed to evaluate the stability of analyte in processed samples. The samples of the accepted precision and accuracy batch were re-analyzed after 48 hours and the results were within acceptance criteria. The % CV of the quality control samples ranged between 1.31 to 10.95 %. The percentage change (%Bias) of the nominal concentration across quality control samples ranged in between -5.83% to 2.28%. The results are presented in Table 6

Sample Concentration	Mean conc. found (ng/mL)	Precision (CV%,n = 6)	Mean % Change from nominal
Plasma sample ambient storage (18 h)			
LQC: 6.00 ng/mL	5.86	2.18	-2.28
HQC: 800.00 ng/mL	830.14	1.59	3.77
Freeze-thaw cycles (n = 3) at -70°C			
LQC: 6.00 ng/mL	5.94	5.02	-0.97
HQC: 800.00 ng/mL	819.75	1.89	2.47
Freeze-thaw cycles (n = 3) at -20°C			
LQC: 6.00 ng/mL	6.07	2.92	1.22
HQC: 800.00 ng/mL	800.75	2.88	0.09
Long-term stability in plasma at -70°C for 15 days			
LQC: 6.00 ng/mL	5.87	4.4	-2.22
HQC: 800.00 ng/mL	800.17	2.2	0.02
Long-term stability in plasma at -20°C for 15 days			
LQC: 6.00 ng/mL	5.94	2.2	-1.06
HQC: 800.00 ng/mL	802.73	4.02	0.34

**Table 5. Stability studies of rilpivirine in Sprague Dawley rat serum**

QC sample nominal concentration (ng/mL)	Intra – day			
	LLOQ	LQC	MQC	HQC
	2.00	3.00	500.00	800
N	6	6	6	6
Mean	1.99	5.65	511.41	806.74
%CV	7.52	10.95	1.31	2.90
%Bias	-0.33	-5.83	2.28	0.84

Table 6. Re-injection reproducibility for 48 hours at 4°C

### 3.6. Application

All experiments in the current study conformed to the committee for the purpose of control and supervision on experiments on animals (CPCSEA). Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals. They were used to collect the samples and then sacrificed according to the Criteria for use of Laboratory Animals provided by the CPCSEA resolution on the use of animals in research. The method was successfully used to perform the determination of serum concentrations of rilpivirine after oral administration of 5 mg/kg dose to 6 males Sprague Dawley rats. The protocol of bioequivalence study was approved by the US Food and Drug Administration. Experimental pharmacokinetic determinations were accomplished by using 6 healthy males Sprague Dawley rats, weighing 200±20 gms. The animals were housed in groups of three under standard laboratory conditions of constant temperature (21±2°C) and a 12/12 h light/dark cycle for at least 7 days prior to testing. Commercial food pellets and tap water was freely available. Animals were transferred to the testing laboratory at least one day before the start of the experiments. No food was supplied in the last 12 hours before the experiment.

Doses were established depending on the linearity of pharmacokinetic parameters in rat and the necessary conditions requested by the analytical method, thereby 5 mg/kg, orally to each group of rats. 500 µL blood samples were drawn from the femoral artery at 0, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hrs and transferred into eppendorf tubes. Blood samples were immediately centrifuged at 4000 rpm for 20 min. The clear serum layer was transferred to clean test tubes and stored at -20 °C for Rilpivirine analysis in each serum samples. For the analysis, aliquots of 0.05 ml of serum were supplemented with 10µl of internal standard solution (didanosine 1 µg/ml) and the mixtures diluted as same volume by mobile phase. After dilution the samples were centrifuged at 10000 rpm for 10 min to spin down the precipitate, the supernatant loaded into auto sampler and 20µl were injected into the LC system. The observed mean serum concentration - time profile of rilpivirine following oral administration of 5 mg/kg is

shown in Figure 3. The main pharmacokinetic parameters are summarized in Table 7. The chromatogram of 1 h time point sample was shown in figure 4. All the pharmacokinetic parameters were shown low relative standard deviation, indicating high precision of pharmacokinetic parameters. Rilpivirine reached the maximum serum concentrations at 1.50 h, with serum concentrations of 481.83 ng/mL. According to other published papers [9], *T<sub>max</sub>* of rilpivirine is 2 h, which are similar to our results. We quantified all serum concentrations up to 24 h, despite of low dose (5 mg/kg). The satisfactory precision and percent recovery of standard addition samples indicate that this newly developed and validated method provides a reliable quantification approach for the measurement of rilpivirine in rat serum.

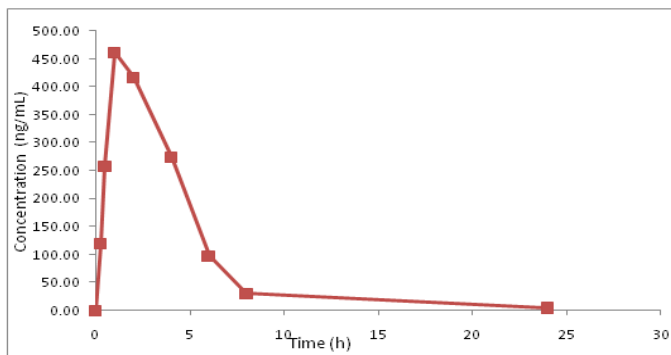


Figure 3. Mean serum concentration - time profile following oral administration of 5 mg/kg

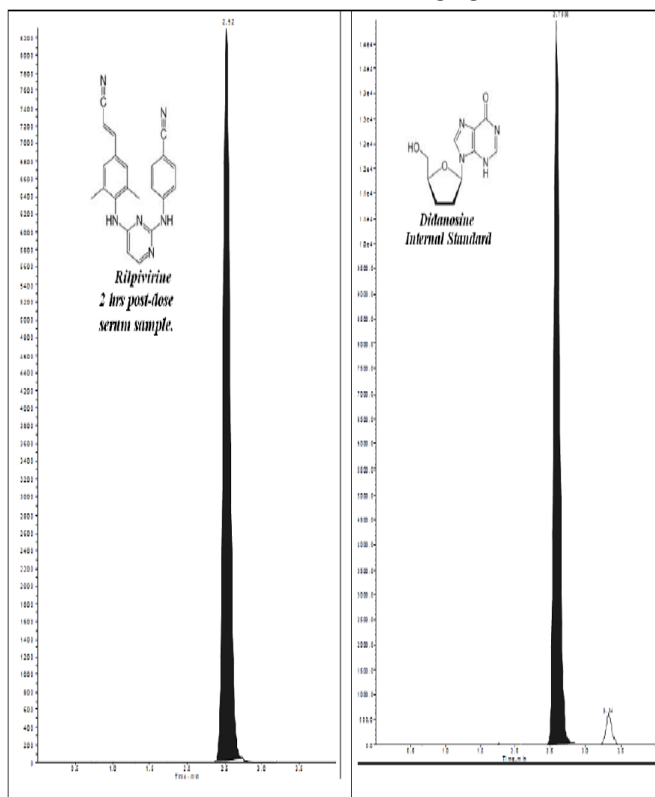


Figure 4: Chromatogram of Rilpivirine in rat serum obtained after 2 hrs post-dose serum sample.



Parameters	Units	Rilpivirin
C max	ng/mL	491.67±23.17
AUC 0–24	ng.h/ml	2153.13±58.40
T max	H	1.50±0.55

**Table 7. Pharmacokinetic parameters (Mean ± S.D.) after single-dose oral administration of rilpivirin in Sprague Dawley rat serum**

#### 4. CONCLUSIONS

A new LC-MS/MS method for the quantification of rilpivirine in Sprague Dawley rat serum was developed. The method was found to be superior in LLOQ compared with other methods. The specificity, sensitivity, accuracy, precision, recovery and stability met the requirements of FDA guidance. The method was successfully applied to a pharmacokinetic study in Sprague Dawley rat serum following oral administration of rilpivirine at 5 mg/kg. The pharmacokinetic parameters for rilpivirine were analyzed by a non-compartment model.

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#### 6. REFERENCES

1. Dluhy, R.G. Effect of inhaled beclomethasone dipropionate and budesonide on adrenal function, skin changes and cataract formation. *Resp. Med. (Suppl. B)*, 1998, 92, 15–23.
2. Chen, B.; Li, M.; Lin, M.; Tumambac, G.; Rustum, A. A comparative study of enol aldehyde formation from betamethasone, dexamethason, beclomethasone and related compounds under acidic and alkaline conditions. *Steroids*, 2009, 74, 30-41.
3. Azijn, H.; Tirry, I.; Vingerhoets, J.; de Bethune, MP.; Kraus, G.; Boven, K.; Jochmans, D.; Van Craenenbroeck, E.; Picchio, G.; Rimsky, LT.; TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. *Antimicrob Agents Chemother.* 2010; 54: 718-727.

4. Ford, N.; Lee, J.; Andrieux-Meyer, I.; Calmy, A.; Safety, efficacy, and pharmacokinetics of rilpivirine: ystematic review with an emphasis on resource-limited settings. *HIV AIDS (Auckl)*. 2011; 3 : 35-44.
5. Wilkin, A.; Pozniak, AL.; Morales-Ramirez, J.; Lupo, SH.; Santoscoy, M.; Grinsztejn, B.; Ruxrunghtham, K.; Rimsky, LT.; Vanveggel, S.; Long-term efficacy, safety, and tolerability of rilpivirine (RPV, TMC278) in HIV type1- infected antiretroviralnaïve patients: week 192 results from a phase IIb randomized trial. *AIDS Res Hum Retroviruses*. 2011; 28 : 437-446.
6. AIDS info Drug Database: Emtricitabine/Rilpivirine/Tenofovir disoproxil fumarate. Available at <http://www.aidsinfo.nih.gov/drugs/441/complera/patient/> Accessed July 5, 2012.
7. Else, L.; Watson, V.; Tjia, J.; Hughes, A.; Siccardi, M.; Khoo, S.; Back, D.; Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010; 878: 1455-1465.
8. Jemal, M.; Xia Y.-Q.; LC-MS development strategies for quantitative bioanalysis. *Cur. Drug Met.* 2006; 7: 491–502.
9. Mathias, A.; Menning, M.; Wiser, L.; Wei, X.; Dave, A.; Chuck, S.; and Kearney, BP.; Bioequivalence of the Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate Single Tablet Regimen. *J Bioequiv Availab* 2012, 4:7
10. U.S. Food and Drug Administration. 2001. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf>
11. Center for drug evaluation and research. Application Number: 202022Orig1s000 Clinical pharmacology and biopharmaceutics review(s). [www.accessdata.fda.gov/drugsatfda.../202022Orig1s000ClinPharmR.pdf](http://www.accessdata.fda.gov/drugsatfda.../202022Orig1s000ClinPharmR.pdf)

**Conflict of Interest: None Declared**

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